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Two new asterosaponins, archasterosides A and B, from the Vietnamese starfish *Archaster typicus* and their anticancer properties

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ABSTRACT

New asterosaponins archasterosides A (**1**), B (**2**), and the known regularoside A (**3**) were isolated from the Vietnamese starfish *Archaster typicus* and structurally elucidated by extensive NMR techniques and chemical transformations. Compounds **1**–**3** showed moderate cytotoxic activities against HeLa and mouse JB6 P^+ Cl41 cell lines. The most active, **2**, induced basal AP-1- and p53-, but not NF-κB-transcriptional activations in JB6 Cl41 cells.

The secondary metabolites from starfish are characterized by a remarkable diversity of different polar steroids, including polyhydroxysteroids and related mono- and biosides as well as toxic steroid oligoglycosides named asterosaponins.[1] Asterosaponins contain 3β,6α-dihydroxysteroid aglycons with a 9(11)-double bond and sulfate group at C-3. Their carbohydrate chains embrace from four to six monosaccharide units attached to C-6 of an aglycon. These glycosides show a wide spectrum of biological activities, for instance cytotoxic, antiviral, antibacterial, antibiofouling, and antifungal effects.[1]

Earlier 10 polyhydroxysteroids from the starfish *Archaster typicus* (order Valvatida, family Archasteridae) were reported.[2] However, no asterosaponins have been isolated to date from this species. In a continuation of our search for new bioactive polar steroidal compounds from starfish,[3] we have examined composition and biological activities of oligoglycoside fraction from this species, collected off Quang Ninh, Vietnam. In this Letter we report the structures of new archasterosides A (**1**) and B (**2**), isolated together with a known regularoside A (**3**), as well as their cytotoxic properties. The ethanol extract of *A. typicus* was subjected to chromatographic separation on columns with Teflon powder Polychrome 1, Si gel, and Florisil. Application of ion-pair HPLC to the obtained oligoglycoside subfractions, using a semipreparative Diasfer-110-C18 ($EtOH/H_2O$/1 M NH_4OAc, 55:44:1) and Discovery C18 ($MeOH/H_2O$/1 M NH_4OAc, 72:24:1 or 72:27:1) columns yielded **1**–**3**.[4] Compound **3** was identified by comparison of the NMR and MS data with literature values as regularoside A, originally isolated from the starfish *Halityle regularis*.[5]

The molecular formula of archasteroside A (**1**) was determined as $C_{59}H_{97}O_{28}SNa$ from the $[M-Na]^-$ molecular anion at *m/z* 1285.5910 (calcd for $C_{59}H_{97}O_{28}S$, 1285.5893) in the negative HRESIMS and the $[M+Na]^+$ sodiated-molecular ion at *m/z* 1331 in the positive ESIMS. The fragment-ion peaks at *m/z* 1211 $[(M+Na)-NaHSO_4]^+$ in the positive ESIMS/MS and at *m/z* 97 $[HSO_4]^-$ in the negative ESIMS/MS exhibited the presence a sulfate group in **1**. The comparison of ^1H and ^{13}C NMR spectra of glycosides **1** and **3** suggested that oligosaccharide moieties in both compounds are identical. In the confirmation, the ^1H NMR spectrum of **1** exhibited five signals of anomeric protons at δ_H 4.91, 4.96, 4.82, 5.01, and 5.25 (with coupling constants 7.3–8.0 Hz) correlating in the HSQC experiment with corresponding carbon resonances at δ_C 105.0, 103.6, 102.3, 106.9, and 105.9, respectively (Table 1). These data indicated the existence of five monosaccharide units, bonded by β-glycosidic with each other and aglycon. The methyl doublets at δ_H 1.71, 1.46, 1.48, and 1.80 in the ^1H NMR spectrum confirmed the presence of four 6-deoxy sugars.

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Table 1
^{1}H and ^{13}C NMR data, HMBC, and NOESY correlations[a] of oligosaccharide moiety of **1** and **2**

C No.	δ_C[b]	δ_H[c] (*J* in Hz)	HMBC (H→C)	NOESY (H→H)
Glc				
1	105.0	4.91, d (7.3)	C-6 of aglycon	H-6 of aglycon; H-3, H-5 Glc
2	73.6	3.97, t (8.2)	C-1 Glc	
3	91.6	3.84, t (8.8)	C-2, C-4 Glc; C-1 Qui^{2}	H-1 Glc
4	69.6	4.04, t (9.6)		
5	77.4	3.82, m		H-1 Glc
6	62.2	4.44, dd (2.5, 12.0) 4.27, dd (5.3, 11.9)		
Qui^{1}				
1	104.8	4.80, d (8.1)	C-6 of aglycon	H-6 of aglycon; H-3, H-5 Qui^{1}
2	73.8	3.95, t (8.1)	C-3 Qui^{1}	
3	91.0	3.76, t (8.9)	C-1 Qui^{2}	H-1 Qui^{1}, H-1 Qui^{2}
4	74.4	3.52, t (9.2)	C-3 Qui^{1}	H-6 Qui^{1}
5	71.7	3.67, m		H-1 Qui^{1}
6	18.2	1.56, d (6.0)	C-4, C-5 Qui^{1}	H-4 Qui^{1}
Qui^{2}				
1	103.6	4.96, d (7.3)	C-3 Glc for **1** or C-3 Qui^{1} for **2**	H-3, H-5 Qui^{2}; H-3 Glc for **1** or H-3 Qui^{1} for **2**
2	82.5	4.07, t (7.9)	C-1 Qui^{2}, C-1 Qui^{4}	
3	75.1	4.11, t (8.8)	C-2 Qui^{2}	H-1 Qui^{2}
4	85.6	3.55, t (8.4)	C-3, C-5 Qui^{2}; C-1 Qui^{3}	H-6 Qui^{2}, H-1 Qui^{3}
5	71.4	3.88, m		H-1 Qui^{2}
6	18.0	1.71, d (6.0)	C-4, C-5 Qui^{2}	H-4 Qui^{2}
Qui^{3}				
1	102.3	4.82, d (8.0)	C-4 Qui^{2}	H-3, H-5 Qui^{3}; H-4 Qui^{2}
2	84.3	3.98, t (8.2)	C-3 Qui^{3}	
3	77.5	4.10, t (9.1)		H-1 Qui^{3}
4	75.7	3.60, t (9.0)	C-3, C-5 Qui^{3}	H-6 Qui^{3}
5	72.8	3.68, m		H-1 Qui^{3}
6	17.7	1.46, d (6.0)	C-4, C-5 Qui^{3}	H-4 Qui^{3}
Fuc				
1	106.9	5.01, d (7.7)	C-2 Qui^{3}	H-2 Qui^{3}; H-3, H-5 Fuc
2	73.7	4.38, dd (7.8, 9.6)	C-1, C-3 Fuc	
3	74.9	4.04, dd (3.9, 9.7)		H-1 Fuc
4	72.3	3.97, d (2.5)		H-6 Fuc
5	71.7	3.77, m	C-4 Fuc	H-1 Fuc
6	16.9	1.48, d (6.5)	C-5 Fuc	H-4 Fuc
Qui^{4}				
1	105.9	5.25, d (7.4)	C-2 Qui^{2}	H-3, H-5 Qui^{4}
2	76.3	4.02, t (8.5)		
3	76.9	4.07, t (8.9)		H-1 Qui^{4}
4	75.5	3.96, t (8.8)		H-6 Qui^{4}
5	73.6	3.69, m		H-1 Qui^{4}
6	17.8	1.80, d (6.5)	C-4, C-5 Qui^{4}	H-4 Qui^{4}

[a] Measured in C_5D_5N.
[b] At 125.8 MHz.
[c] At 500 MHz. Assignments from ^{1}H–^{1}H COSY, HSQC, 1D TOCSY, HSQC–TOCSY, HMBC, and NOESY data.

The carbon signals of the monosaccharide residues in the ^{13}C NMR spectrum of **1** coincided with those of terminal β-D-fucopyranosyl and β-D-quinovopyranosyl units and internal 2-substituted β-D-quinovopyranosyl, 2,4-disubstituted β-D-quinovopyranosyl, and 3-substituted β-D-glucopyranosyl units from the ^{13}C NMR spectrum of regularoside A.[5] The negative ESIMS/MS of a molecular anion at *m/z* 1285 $[M-Na]^-$ registered a series of fragment-ion peaks, confirming the structure of a carbohydrate chain in **1**. There were peaks with *m/z* 1139 $[(M-Na)-146]^-$, 993 $[(M-Na)-2\times 146]^-$, 847 $[(M-Na)-3\times 146]^-$, 701 $[(M-Na)-4\times 146]^-$, due to the losses of one, two, three, and four 6-deoxyhexose units, respectively; and 539 $[(M-Na)-162-4\times 146]^-$ and 521 $[(M-Na)-180-4\times 146]^-$, due to the loss of a carbohydrate chain. The D-configurations of all monosaccharide units of **1** (glucose, fucose, and quinovose) were established by hydrolysis of **1** with 2 M TFA followed by alcoholysis with (*R*)-(−)-octanol, acetylation, and determination of GC retention times of octyl derivatives according to the procedure of Leontein et al.[6] All proton and carbon signals attributable to the carbohydrate moiety of **1** were assigned by the application of 1D and 2D NMR experiments including ^{1}H–^{1}H COSY, HSQC, TOCSY, HSQC–TOCSY, HMBC, and NOESY (Table 1). The positions of interglycosidic linkages and attachment of the carbohydrate chain to steroidal aglycon were deduced from NOESY and HMBC spectra, where cross-peaks between H-1 of Glc and H-6 (C-6) of the aglycon, H-1 of Qui^{2} and H-3 (C-3) of Glc, H-1 of Qui^{3} and H-4 (C-4) of Qui^{3}, H-1 of terminal Qui^{4} and C-2 of Qui^{2}, and H-1 of terminal Fuc and H-2 (C-2) of Qui^{3}, respectively, were observed.

The NMR data of steroidal aglycon of **1** were shown to be related to those of regularoside A[5] and differed from **3** only in the substitution of the Me group at C-24 in the side chain on the Et group, that agreed with the molecular mass difference of 14 amu between **1** and **3**. The ^{1}H, ^{13}C NMR and DEPT spectra of **1** (Table 2) revealed proton and carbon signals of two angular Me groups (δ_H 1.00, 0.95; δ_C 13.2, 19.1), a 9(11)-double bond (δ_H 5.26; δ_C 145.6, 116.4), one methine group (δ_H 4.89, δ_C 77.3), bearing a *O*-sulfate group, and one methine group (δ_H 3.81, δ_C 80.5), bearing a *O*-carbohydrate chain, characteristic for steroidal nucleus of asterosaponins.[5] The proton and carbon signals of aglycon side chain showed the presence one tertiary Me group (δ_H 1.45, δ_C 23.5), one primary Me group (δ_H 0.95, δ_C 12.2), two secondary Me groups

Table 2
^{1}H and ^{13}C NMR data[a] for aglycon moieties of **1** and **2**

C No.	1		2	
	δ_C[b]	δ_H[c] (*J* in Hz)	δ_C[b]	δ_H[c] (*J* in Hz)
1	35.9	1.66, m 1.41, m	35.9	1.68, m 1.41, m
2	29.4	2.78, m 1.89 m	29.4	2.79, m 1.89 m
3	77.3	4.89, m	77.4	4.89, m
4	30.7	3.47, m 1.70, m	30.7	3.45, d (11.2) 1.70, m
5	49.2	1.52, m	49.3	1.50, m
6	80.5	3.81, m	80.2	3.80, dt (4.4, 10.8)
7	41.3	2.70, m 1.25, m	41.3	2.71, dt (5.0, 12.6) 1.29, q (11.5)
8	35.2	2.06, m	35.0	2.26, m
9	145.6		145.7	
10	38.2		38.2	
11	116.4	5.26, m	116.5	5.26, d (5.8)
12	42.2	2.31, m 2.09, m	42.9	2.34, dd (6.4, 16.3) 2.00, m
13	41.7		41.6	
14	53.7	1.19, m	52.0	1.14, m
15	25.0	1.67, m 1.16, m	38.9	2.46, dt (7.5, 12.7) 1.65, m
16	22.9	1.86, m 2.13, m	73.2	4.87, m
17	59.5	1.70, m	60.4	1.38, d (7.0)
18	13.2	1.00, s	14.8	1.42, s
19	19.1	0.95, s	19.2	0.97, s
20	71.2		76.0	
21	23.5	1.45, s	26.3	1.47, s
22	64.6	2.89, d (2.3)	44.7	1.96, m
23	56.0	2.96, dd (2.2, 8.5)	22.5	1.62, m 1.48, m
24	48.4	1.01, m	40.0	1.20, m
25	29.7	1.88, m	27.9	1.55, m
26	19.7	0.97, d (6.1)	22.6	0.88, d (6.5)
27	19.3	0.96, d (6.1)	22.5	0.87, d (6.5)
28	21.2	1.34, m		
29	12.2	0.95, t (7.8)		

[a] Measured in C_5D_5N.
[b] At 125.8 MHz.
[c] At 500 MHz. Assignments from ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY data.

(δ_H 0.97, 0.96; δ_C 19.7, 19.3), and a 22,23-epoxy group [δ_H 2.89 (d, 2.3), 2.96 (dd, 2.2, 8.5); δ_C 64.6, 56.0]. Based on these data, a 22,23-epoxy-24-ethyl-5α-cholest-9(11)-ene-3β,6α,20-triol aglycon with glycoside linkage at C-6 and a *O*-sulfate group at C-3 has been assumed. The 20*R*-configuration was expected on the basis of the chemical shift of H_3-21 at δ 1.28 (δ 1.28 for 20*R*- and δ 1.13 for 20*S*-hydroxy-22,23-epoxysteroids, CD_3OD).[5,7] The observed NOESY correlations of the side chain protons (Fig. 1) presumed only two variations of the stereochemistry of asymmetric centers C-22, C-23, and C-24 as 22*R*,23*S*,24*S* or 22*S*,23*R*,24*R* from eight possible varieties. Detailed comparison NMR spectra of regularoside A and **1**, registered in CD_3OD, showed that the C-17, C-20, C-21, C-22, H_3-21, H-22, and H-23 signals were similar, but C-23, C-24, and C-25 signals were shifted (from δ 58.8 to 57.4, from δ 42.9 to 49.7, and from δ 32.6 to 30.7, respectively) in accordance with γ- and β-effects due to the presence of additional methyl group at C-28 in **1**.[5,7] We are of the opinion the 20*R*,22*R*,23*S*,24*S* configuration for **1** are the most preferable by analogy with natural regularoside A, in which the same configuration was ascertained by comparison with model compounds.[5] Hence, the structure of archasteroside A was elucidated as **1**. The new asterosaponin **1** contains a 22,23-epoxy-20-hydroxy-stigmastane side chain never found in other asterosaponins earlier.

The molecular formula of archasteroside B (**2**) was established as $C_{57}H_{95}O_{27}SNa$ from the $[M-Na]^-$ molecular anion at *m/z* 1243.5796 (calcd for $C_{57}H_{95}O_{27}S$, 1243.5787) in the negative HRESIMS and the $[M+Na]^+$ sodiated-molecular ion at *m/z* 1289 in the positive ESIMS. The examination of the NMR spectra and extensive 2D NMR studies of glycosides **2** and **1** indicated that both compounds possess the related pentasaccharide moieties differed in the replacement of the 3-substituted β-D-glucopyranosyl residue at C-6 of aglycon on the 3-substituted β-D-quinovopyranosyl unit (Table 1). The carbon signals of the last monosaccharide matched to those of the 3-substituted β-D-quinovopyranosyl residue from the reported spectra of known asterosaponins.[5] In the negative ESIMS/MS of the molecular anion at *m/z* 1243 $[M-Na]^-$ a series of fragmentations with the losses of one, two, three, four, and five 6-deoxyhexose units, respectively, at *m/z* 1097 ($[(M-Na)-146]^-$), 951 ($[(M-Na)-2\times146]^-$), 805 ($[(M-Na)-3\times146]^-$), 659 ($[(M-Na)-4\times146]^-$), 513 ($[(M-Na)-5\times146]^-$), and 495 ($[(M-Na)-164-4\times146]^-$), were observed. NOESY and HMBC experiments allowed us to establish the positions of the glycosidic linkages and connection of the carbohydrate chain to steroidal aglycon. The cross-peaks between H-1 of Qui^1 and H-6 (C-6) of the aglycon, H-1 of Qui^2 and H-3 (C-3) of Qui^1, H-1 of Qui^3 and H-4 (C-4) of Qui^2, H-1 of terminal Qui^4 and C-2 of Qui^2, and H-1 of terminal Fuc and H-2 (C-2) of Qui^3, respectively, were detected. We presumed the D-configuration for all units of fucose and quinovose by analogy with co-occurring asterosaponins **1** and **3**.

The ^{1}H and ^{13}C NMR data of aglycon moiety of glycoside **2** were similar to those of glycoside **1** and showed the presence

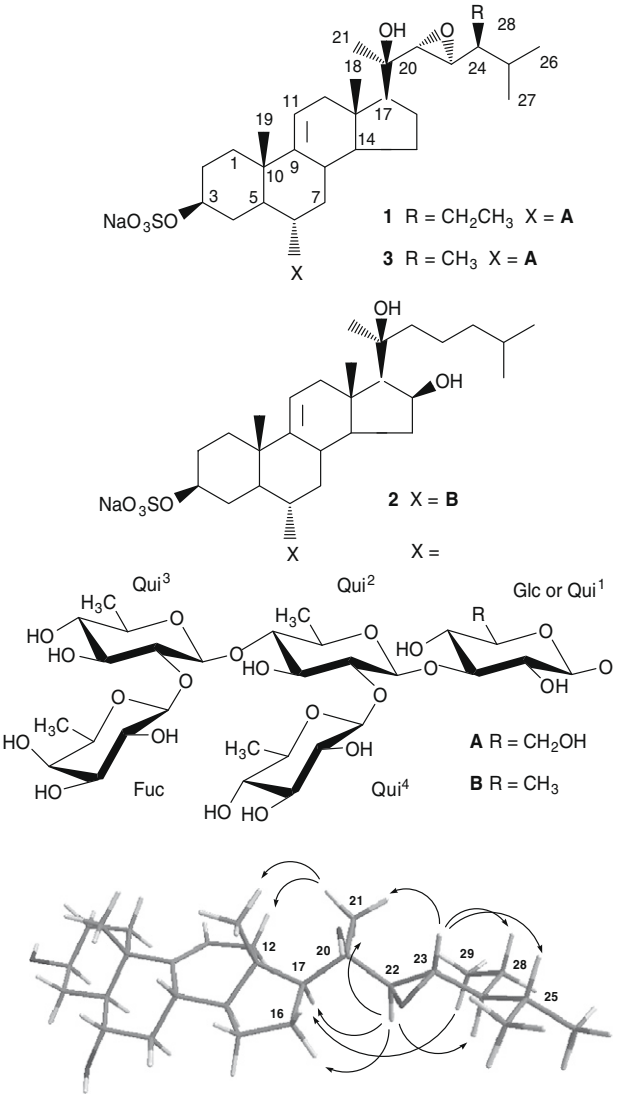

Figure 1. Selected NOESY correlations of the aglycon moiety of archasteroside A (**1**).

of the same $\Delta^{9(11)}$-3β,6α-dihydroxy steroidal nucleus with sulfate group linked at C-3, carbohydrate moiety linked at C-6 and 20-hydroxy cholestane side chain lacking 22,23-epoxy functionality (Table 2). However, the signals of four carbons C-14, C-15, C-16, C-18, and protons H_3-18 in the NMR spectra of **2** differed from the same signals of **1**. The carbon and proton signals of Me-18 were downfield shifted from δ_C 13.2 to 14.8 and from δ_H 1.00 to 1.42, respectively. Along with the MS data these evidences allowed us to suppose the presence of an additional 16β-hydroxy group located in the ring D. The assignments of the NMR signals associated with aglycon moiety were derived from ^1H–^1H COSY, HSQC, HMBC, and NOESY experiments and coincided with those observed in downeyoside K, the glycoside containing the same (20*S*)-5α-cholest-9(11)-en-3β,6α,16β,20-tetraol aglycon, found in the starfish *Henricia downeyae*.[8] The 20*S*-configuration was assumed on the basis of the NOESY correlation of H-21/H-12 as well as chemical shift of H-21 at δ 1.47, which was consistent with that of known asterosaponins.[8,9] Thus, the structure of archasteroside B was defined as **2**. The carbohydrate moiety of **2** composed only from β-D-fucopyranosyl and β-D-quinovopyranosyl units is reported in asterosaponins for the first time. The steroidal moiety of **2** never has been found in asterosaponins because downeyoside K embracing the same steroidal part belongs to other series of steroid glycosides. It possesses the sulfate group at C-6 and β-D-glucuronopyranosyl unit at C-3 of aglycon in contrast with 'classical' asterosaponins which have the sulfate at C-3 and the oligosaccharide moiety at C-6 of aglycon.

The in vitro cytotoxicity of **1**–**3** was evaluated by 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)-tetrazolium, inner salt (MTS) assay.[10] The asterosaponins **1**, **2**, and **3** demonstrated moderate anticancer activity and showed cytotoxic effects against human cancer HeLa cells with IC_{50} = 24, 14, and 110 μM, whereas against mouse epidermal JB6 P^+ Cl41 cells demonstrated cytotoxic effects with IC_{50} = 37, 18 μM, and **3** was inactive up to 50 μM concentration, respectively. The action of asterosaponin **2**, the most active among the compounds studied, on the basal AP-1-, p53-, and NF-κB-dependent transcriptional activities was also examined using JB6 Cl41 cells stably expressing a luciferase reporter gene controlled by an AP-1, p53, or NF-κB DNA binding sequence.[11] As was shown, after 6 h of incubation

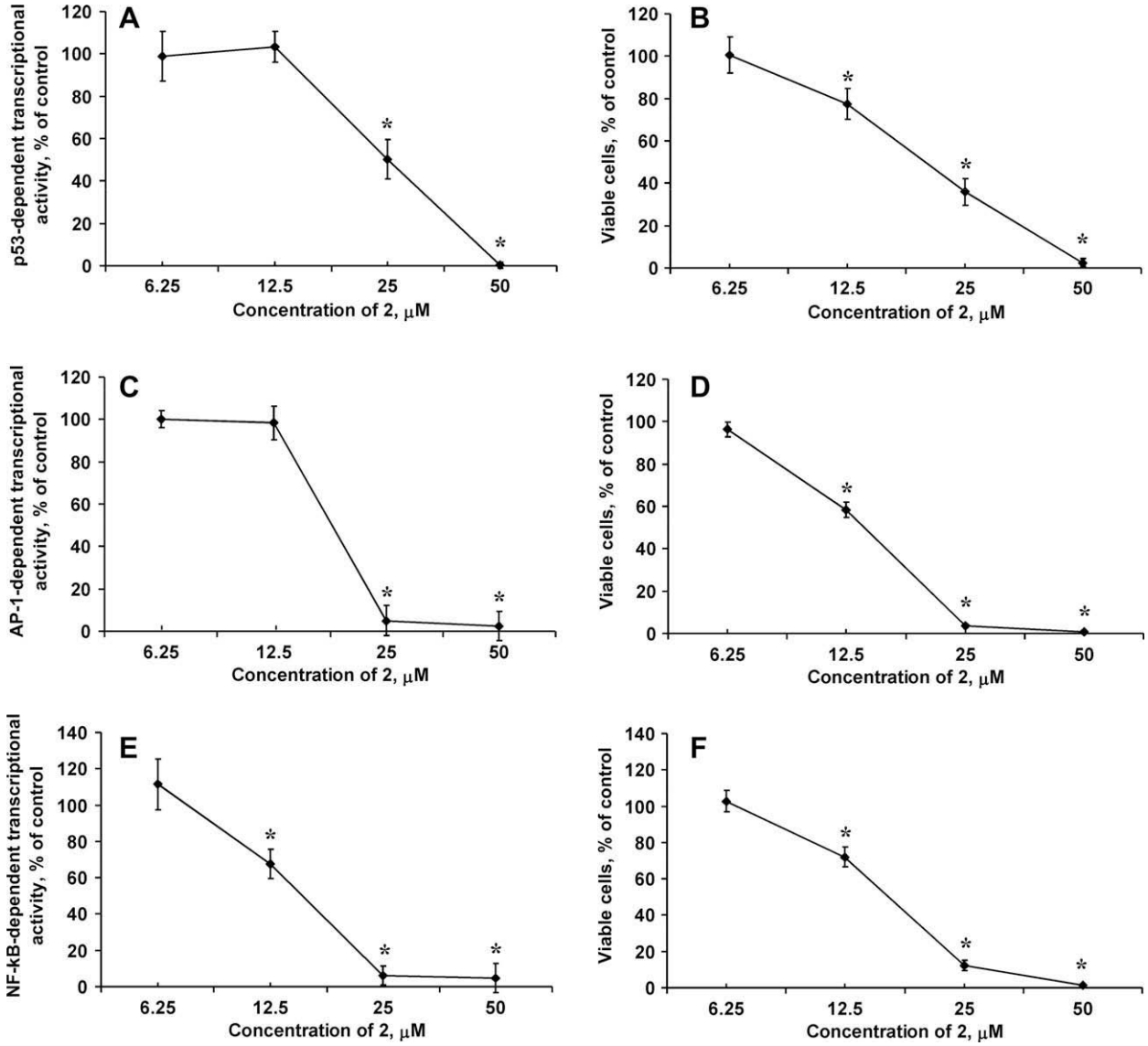

Figure 2. Effects of asterosaponin **2** on the basal p53 (A), AP-1 (C), or NF-κB (E)—dependent transcriptional activity in JB6 Cl41 mouse epidermal cells evaluated after 6 h of the treatment. Effects of asterosaponin **2** on the viability of JB6 Cl41 p53 (B), AP-1 (D), or NF-κB (F) cells evaluated after 6 h of the treatment. Data are represented as means ± SD of six samples from two independent experiments. The asterisk (*) indicates a significant distinction (p <0.05) in the nuclear factor activation, or in JB6 Cl41 cells viability compare to control.

with the cells, **2** at 12.5 μM concentration 1.3 and 1.7 times as much induced basal p53- and AP-1-, but not NF-κB-dependent transcriptional activities compared to untreated control cells (Fig. 2). To our best knowledge this is the first report about asterosaponins inducing p53- and AP-1-dependent transcriptional activities.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.005.

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4. *Extraction and isolation*. The fresh animals (7 kg) were chopped and extracted three times with EtOH at 20 °C. The H_2O/EtOH layer was evaporated, and the residue was dissolved in H_2O (1 L). The H_2O-soluble fraction was passed through a Polychrome 1 column (7 × 26 cm) and eluted with distilled H_2O until a negative chloride ion reaction was obtained, followed by elution with EtOH. The combined EtOH eluate was evaporated to give a brownish material (29.7 g). The resulting total fraction of steroidal compounds was chromatographed on a Si gel column (6.5 × 20 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1→1:6) to give fractions 1–9. Fraction 7 (370 mg), containing sulfated polyhydroxysteroids and asterosaponins, was separated on a Florisil column (4 × 17 cm) using $CHCl_3$/EtOH (stepwise gradient, 4:1→1:2) to give fractions 7.1–7.3. Fraction 7.3 (95 mg), containing mainly asterosaponins, was purified by HPLC on Diasfer-110-C18 column (10 μm, 250 × 15 mm, 2.5 mL/min) using EtOH/H_2O/1 M NH_4OAc (55:44:1) to give fractions 7.31 (t_R 31.5 min) and 7.32 (t_R 48.7 min), and pure **1** (4.1 mg, t_R 51.1 min, colorless amorphous powder, $[\alpha]_D^{20}$ +4.8 (*c* 0.2, MeOH)). Fraction 7.31 (12 mg) was purified by HPLC on Discovery C18 column (5 μm, 250 × 10 mm, 2.5 mL/min) using MeOH/H_2O/1 M NH_4OAc (72:27:1) to yield **3** (2.8 mg, t_R 18.8 min), fraction 7.32 (6 mg) was purified on the same column using MeOH/H_2O/1 M NH_4OAc (75:24:1) to yield **2** (3.7 mg, t_R 17.4 min, colorless amorphous powder, $[\alpha]_D^{20}$ +9.8 (*c* 0.3, MeOH)).
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7. ^{1}H NMR (aglycon side chain of **1**, 700 MHz, CD_3OD): 1.28 (s, H_3-21), 2.74 (d, *J* = 2.3 Hz, H-22), 2.76 (dd, *J* = 2.4, 8.6 Hz, H-23), 0.90 (m, H-24), 1.87 (m, H-25), 0.95 (d, *J* = 7.0 Hz, H_3-26), 0.99 (d, *J* = 7.0 Hz, H_3-27), 1.35 (m, H-28), 1.43 (m, H-28′), 0.94 (t, *J* = 7.5 Hz, H_3-29). ^{13}C NMR (aglycon side chain of **1**, 176.0 MHz, CD_3OD): 72.6 (C-20), 23.5 (C-21), 65.5 (C-22), 57.4 (C-23), 49.7 (C-24), 30.7 (C-25), 19.9 (C-26), 19.6 (C-27), 22.1 (C-28), 12.6 (C-29).
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